Declaration of Dr. Jean-Marie Saint-Remy under 37 CFR § 1.132

1. I, Jean-Marie Saint-Remy declare and state as follows. I am a professor at the University of Leuven and I am an expert in the field of vascular biology. I am one of the inventors of the U.S. Patent Application No. 10/044,569 entitled 'Method and pharmaceutical composition for preventing and/or treating systemic inflammatory response syndrome'.
2. I understand that the Examiner has questioned, in the Office Action mailed on April 21st, 2004, the fact that the in vitro effect observed with monoclonal anti-FVIII CI domain antibodies on thrombin formation, is enabling for an in vivo therapeutic use to prevent and/or treat systemic inflammatory response syndrome, such as seen in sepsis.
3. I herewith present additional data which directly demonstrate the ability of a monoclonal anti-FVIII CI domain antibody, which partially inhibits FVIII, to prevent LPS-induced sepsis *in vivo*.
4. In our laboratory, an scFv of KRIX-1 was constructed by adding a linker sequence between the 3' end of the KRIX-1 light chain variable part (VL) and the 5'end of the heavy chain variable part (VH), using standard technology known to the skilled person at the filing date of the present application. The technical details are provided herewith. The resulting scFv-KRIX-1VLVH was cloned into the pPICZαC expression vector and the final pPICZαC-scFv-KRIX-1VLVH(His) vector was used to transform X33 cells for scFv production. The supernatant was tested to demonstrate the presence of a functional scFv fragment. The scFv fragment was purified and after concentration, the scFvKRIX-1VLVH(His) was tested in a FVIII chromogenic assay to evaluate the ability of the scFvKRIX-1VLVH(His) to inhibit FVIII activity. The FVIII inhibitory capacity was evaluated in a Besthesda assay (described in the application as filed): one volume of buffer with scFvKRIX-1VLVH(His) at various concentrations was mixed with one volume of a pool of normal human plasma and incubated for 2h at 37°C. The residual FVIII activity was then measured in a chromogenic assay.
5. The results, presented in Figure 1 enclosed herewith, clearly demonstrate that scFv of the Krix-1 antibody has inhibitory activity on FVIII.
6. I hereby declare that all statements made herein are true and are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonement, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Date: 22-11-04

By: Dr. Jean-Marie Saint-Remy

Figure 1.

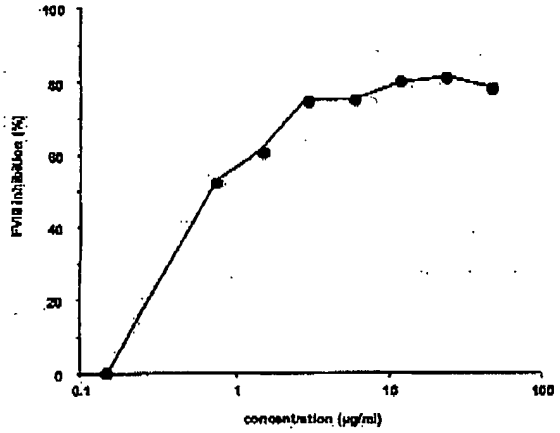


Figure 1: Graph of experimental results showing the FVIII inhibitory activity of scFv fragment of KRIX-1 (scFv-KRIX-1VLVH(His)) produced in Pichia pastoris, in accordance with an embodiment of the invention.

Technical details of the production and characterization of KRIX-1 scFv fragment

Prevention of septic shock with anti-FVIII antibodies

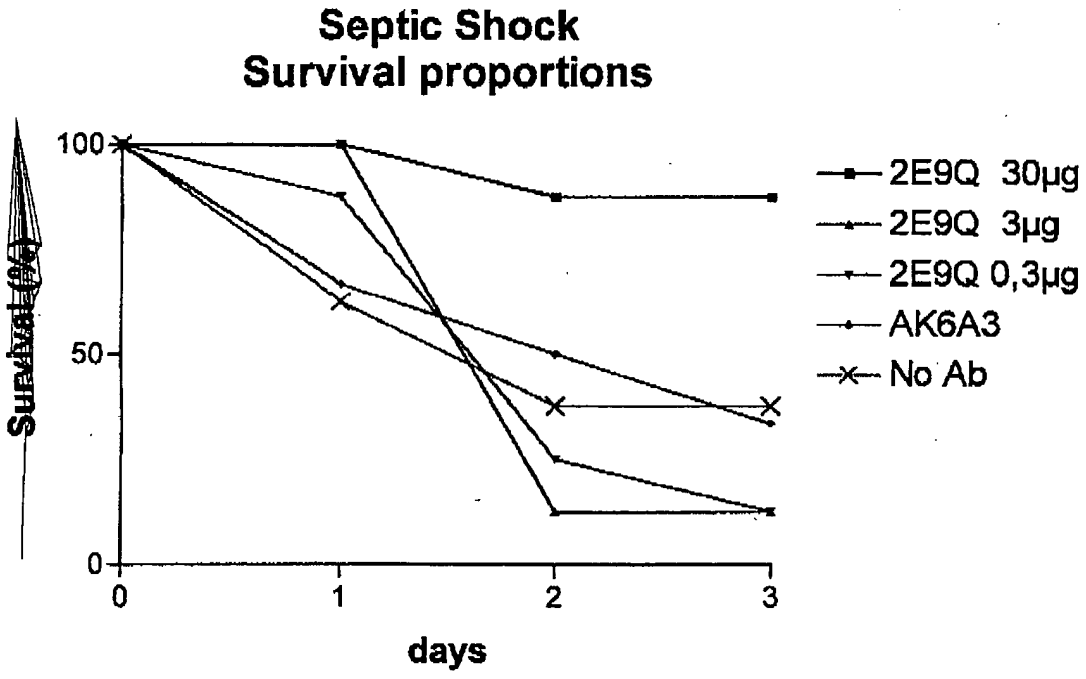
Experiments carried out with LE2E9Q

The maximum plateau inhibition is ±25%. However, this seems to be sufficient for a successful prevention of LPS-induced septic shock.

Mouse model

Wildtype C57Bl/6 mice were injected with LE2E9, LE2E9Q, a sham IgG4 antibody or buffer. Thirty minutes later a single IP injection of 400 μg LPS was made. Mice were followed up for survival.

Results



The Figure shows a highly significant death prevention with 30 μg of LE2E9Q as compared to no antibody (Kaplan-Mayer test, $p < 0.03$), an

irrelevant IgG4 antibody ($p < 0.03$), or to 3 and 0.3 μg LE2E9Q ($p < 0.004$ and $p < 0.003$, respective